Genetic Enhancement of Cold Tolerance of *Cymbidium* by Introducing ω-3 Fatty Acid Desaturase Gene

Zang-Kual U., In-Sup So, Ok-Young Lee-stadelmann, Kyung-Ae Hong, Sung-Jun Song

Cheju Applied Radioisotope Research Institute, Cheju National University, Cheju 690-756

ABSTRACT

Protocorm like bodies(PLBs) were micro-sectioned by a vibratome, and the conditions for *in vitro* culture were optimized. PLBs were induced from the meristem of *Cymbidium* side-bud and proliferated on Kyoto medium (4 ml/liter of Hyponex) supplemented with 1 mg/liter of α-naphthaleneacetic acid (NAA). The good formation of adventitious PLBs was made from 400μm thick micro-sectioned PLBs. The growth and proliferation of PLBs reformed was most effective in Kyoto medium containing 2 or 4 ml/liter of Hyponex and 1.0 mg/liter of NAA and the shoot formation was promoted by the addition of 1 mg/liter of benzyl adenine(BA). As transient expression of β-glucuronidase(GUS) gene could be identified in the bombarded PLB cells, the micro-sectioned PLBs were bombarded with the microprojectiles coated with the plasmid pBIVA1 containing NPTII and *fad7*. Now the cold tolerant transformants of reformed PLBs are being selected on the kanamycin medium.

[Supported by grants from MOE]

INTRODUCTION

Cymbidiums are the tropical/subtropical plants and require the growth temperature of higher than 15℃. Although Cheju is the most suitable place for the orchid cultivation in Korea, temperature often drops below 15℃ during winter. Therefore, even in the green houses heating is needed for their good growth during winter. This makes the growers pay an additional farming expenses, weakening the marketing competition of Cheju orchids.

ω-3 Fatty acid desaturase converts linoleic acid(18 : 2 ω6, 9) to linolenic acid(18 : 3 3, ω6, 9) which is closely related to cold tolerance. Recently, a cDNA(*fad7* gene) encoding the chloroplast ω-3 fatty acid desaturase was isolated by Iba *et al.* (1993) and introduced into tobacco plants by *Agrobacterium tumefaciens* to produce cold tolerant transformant in which the level of trienoic fatty acids is considerably increased (Kodama *et al.* 1994).

The present work aims at genetical enhancing cold tolerance of *Cymbidium* by introducing chloroplast ω-3 fatty acid desaturase gene by biolistic bombardment.

MATERIALS AND METHODS

Plant materials

The meristems isolated aseptically from *Cymbidium* side-bud were used to induce protocorm like bodies(PLBs) on Kyoto medium (pH 5.0-5.3) containing 2 ml/liter Hyponex, 8 g agar, 2 g peptone, 20 g/liter sucrose and 1 mg/liter α-naphthaleneacetic acid(NAA) at 25℃ with 16 hours photoperiods under 1500 lux fluorescent light. PLBs were proliferated on Kyoto liquid medium and subcultured every 3 week.

PLB microsection

PLBs mounted in a styrofoam were cut in the various thickness(200, 400, 800 μm) by a vibrating microtome (Vibratome, Lancer, Model 1000, USA). The microsections were transferred to petridish containing Kyoto solid medium.

Biolistic bombardment

Plasmid DNA(pBIVA1, pBI121) isolated by Wizard Megapreps DNA purification kit(Stratagene, USA) was precipitated on tungsten microcarriers(average diameter 1.1 μm) as described by Wilmink *et al.*(1992). Ten microsectioned PLBs per petridish were bombarded with the DNA coated tungsten particles two times using biolistic particle delivery system(Biorad, PDS-1000/He, USA).

Assay for GUS expression

β-Glucuronadase activity was determined by a histochemical assay(Nishihara *et al*., 1993)

RESULTS AND DISCUSSION

The best growth of PLBs was obtained from the liquid culture medium containing 4 ml/liter of Hyponex and 1 mg/liter of NAA(plate 1).

Plate 1. Protocorm like bodies(PLBs) liquid-cultured for 20 days. PLBs were proliferated on Kyoto medium(pH 5.0~5.3) containing 4 ml/liter Hyponex, 8 g agar, 2 g peptone, 20 g/liter sucrose and 1 mg/liter α-naphthaleneacetic acid (NAA) at 25℃ with 16 hours photoperiods under 1500 lux fluorescent light.

Since it is desirable to cut PLBs as thin as possible not only to avoid the chimeric transformation but also to increase the transformation efficiency, the optimal thickness of PLB sections was checked up and found to be 400 μm(plate 2, 3) ; PLB reformation rate from 200 μm sections was very low but 400 μm and 800 μm gave 74% and 77% of reformation rate respectively.

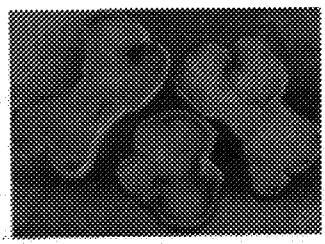

Plate 2. Protocorm like bodies micro-sectioned by vibratome. PLBs mounted in a styrofoam were cut in the 400 μm in thickness by a vibrating microtome (Vibratome, Lancer, Model 1000, USA).

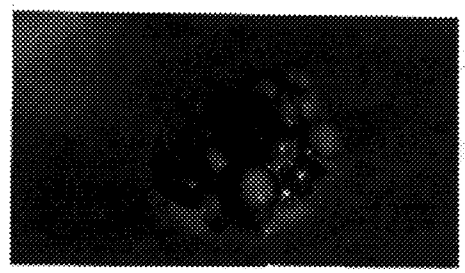

Plate 3. New protocorm like bodies from micro-sectioned protocorm like bodies. The microsections were cultured on Kyoto medium(pH 5.0~5.3) containing 2 ml/liter Hyponex, 8 g agar, 2 g peptone, 20 g/liter sucrose and 1 mg/liter α-naphthaleneacetic acid (NAA) at 25℃ with 16 hours photoperiods under 1500 lux fluorescent light.

From the experiment of NAA/BA combination treatments and Hyponex levels, the proper composition of culture medium for PLB reformation was found to be 2 or 4 ml/liter of Hyponex supplemented with 1.0 mg/liter of NAA and 0.1 mg/liter of BA. One mg/liter of BA with 1.0 mg/liter NAA in Kyoto medium stimulated shoot formation as shown in plate 4.

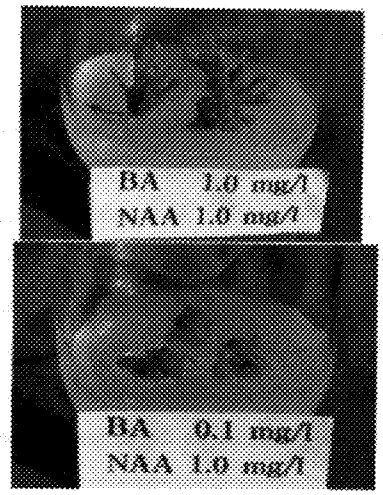

Plate 4. Effect of NAA and BA on growth of protocorm like bodies(PLBs) reformed from the microsectioned PLBs and on their shoot formation.

For the preparation of biolistic bombardment the plasmid pBIVA1 was purified by Wizard Megapreps DNA purification kit, and its purity was proved by electrophoresis(plate 5).

By the histochemical assay technique of GUS expression, the conditions of microprojectile bombardment for PLBs were optimized as 28 inch Hg of chamber vacuum, 0.25 inch of gap distance, 8 mm of microcarrier travel distance and 6 cm of target distance(plate 6). The micro-sectioned PLBs were bombarded

with the microprojectiles coated with the plasmid pBIVA1 containing NPTII and *fad7* and we are now selecting the cold tolerant transformants from newly formed PLBs on the kanamycin medium.

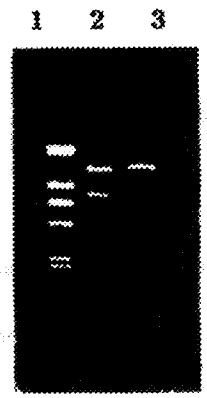

Plate 5. Electrophoresis of pBIVA1 purified by Wizard Megapreps DNA purification kit.
lane 1 : Molecular marker(lamda DNA/Hind Ⅲ)
lane 2 : pBIVA1 undigested
lane 3 : pBIVA1 digested by Sma I

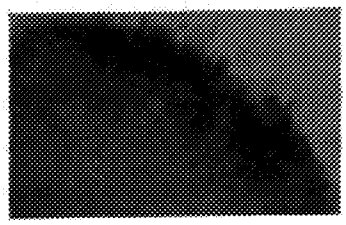

Plate 6. GUS expression in the microsectioned protocorm like body. Plasmid DNA(pBI121) was precipitated on tungsten microcarriers(average diameter 1.1 μm) as described by Wilmink *et al.*(1992). The microsectioned PLBs were bombarded with the DNA coated tungsten particles two times using biolistic particle delivery system(Biorad, PDS-1000/He, USA). β-Glucuronidase activity was determined by a histochemical assay(Nishihara *et al.*, 1993)

REFERENCES

1. Kodama, H., T. Hamada, G. Horiguchi, M. Nishimura and K. Iba(1994) Plant Physiol. 105 : 601－605
2. Iba, K., S. Gibson, T. Nishiuch, T. Fuse, M. Nishimura, V. Arondel, S. Hugly and C. Somerville (1993) J. of Biological Chemistry 268(32) : 24099－24105
3. Wilmink, A., B.C.E. van de Ven and J.J.M. Dons(1992) Plant Cell Repoerts 11 : 76－80
4. Nishihara, M., M. Ito, I. Tanaka, M. Kyo, K. Ono, K. Irifune and H. Morikawa(1993) Plant Physiol. 102 : 357－363